PATENT SPECIFICATION

778,142

Date of Application and filing Complete Specification: Nov. 19, 1954.

No. 33585/54.

Application made in Denmark on Nov. 20, 1953.

Complete Specification Published: July 3, 1957.

Index at Acceptance :—Classes 2 (3), U4 (A1 : A2 : C1 : C4 : C5 : C9 : X), U (5 : 7) ; and 2 (5), R9P.

International Classification :—C07c, f, C08g.

COMPLETE SPECIFICATION

High-Molecular Weight Derivatives of Hydroxyl Group-Containing Steroids and a method of producing them.

We, AKTIEBOLAGET LEO, a Swedish company, of 166, Långvinkelsgatan, Hälsingborg, Sweden, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to high-molecular weight derivatives of hydroxyl group-containing steroids, i.e. steroids with hydroxyl groups in the molecules as well as steroids in which the hydroxyl groups result from enolizing of keto groups.

The invention has for its particular object such derivatives which are able to produce and maintain in the organism, the biological effect of the steroid contained in the derivative for a longer time than it has hitherto been possible. The invention is of particular interest in relation to steroids having a hormonal effect.

It is known that at any time the hormone content in a hormone-producing organ is low in comparison with the amount of hormone which must be supplied to the organism in order to obtain a distinct hormonal effect. This has been substantiated by animal tests as well as by the clinical use of the hormones in question. Therefrom, the conclusion can be drawn that hormones are excreted continuously from the seats of production, and that they do not accumulate there. Accordingly, the best manner of administering hormones should be to imitate as far as possible this natural biologic state of affairs, and to this end it has been tried to produce hormone preparations with a continuous and protracted effect.

The experiments, which have hitherto been made with a view to obtaining a protracted effect of steroid hormone preparations, may be summed up in the following three groups:

(a) Administration of derivatives, mainly esters, as for instance testosterone propionate and estradiol benzoate, where the prolonged effect is produced by the substance having to be hydrolyzed in the organism before a biological effect can set in.

(b) Administration of the hormone in oil depots wherefrom it is only slowly resorbed.

By combining (a) and (b), preparations can be produced which combine both principles.

(c) Administration of the hormone in solid amorphous form as a suspension or an adsorbate on for instance charcoal or aluminium phosphate, by injecting a suspension of microcrystals or by implanting tablets of microcrystals, the so-called pellets.

None of the said methods are particularly satisfactory. Thus injection of the hormone in oil must be repeated fairly often which involves that unpleasant oil depots are left, of which the organism can only difficultly or not at all dispose. Preparations containing steroid hormones adsorbed on for instance aluminium phosphate produce inflammatory reactions and other secondary effects. By implantation of the so-called pellets, an uneven and discontinuous progress of the resorption process is often noticed, and all in all it is questionable whether a sufficiently even and continuous resorption can be obtained in this manner. Moreover, the implantate is often embarrassing, and in some cases it will be expelled from the place of implantation. In respect of the preparations mentioned under (b) and (c) it will further be difficult to insure perfect sterility.

It is an object of the present invention to prepare high-molecular weight compounds of hydroxyl group-containing steroids with sex-hormonal effect, from which compounds it is possible to make preparations for therapeutical purposes which do not have the said disadvantages and in which the hormone has a prolonged effect, in many cases even a very much prolonged effect.

The high-molecular compounds according

[Price 4s 6d]

to the invention are high molecular weight derivatives of hydroxyl group-containing steroids with sex-hormonal effect, including steroids in which the hydroxyl groups result from enolisation of keto groups, said derivatives consisting of molecules, in which the steroid groups present and any aromatic groups which may be present are linked together through phosphate, phosphite or thiophosphate groups, the steroid groups being linked to phosphorus through oxygen atoms and said aromatic groups being linked to phosphorus through oxygen atoms said high molecular weight steroid derivatives being non-dialyzable and soluble in aqueous alkali.

In the following, a more detailed explanation will be given as to the nature of the novel high-molecular weight compounds and the kind of groups contained therein as well as details regarding a preferred method for producing them, the said method also being an object of the invention. Further the effect which can be obtained by means of the novel compounds will be illustrated by means of typical examples.

The high-molecular weight phosphorus containing derivatives of hydroxyl group containing steroids with sex hormonal effect, including steroids with hydroxyl groups resulting from the enolisation of keto groups are produced by reacting steroids having sex hormonal effect and having at least two hydroxyl groups in the molecule, or mixtures of hydroxyl-containing steroids having sex hormonal effect including steroids with hydroxyl groups resulting from the enolisation of keto groups with a coupling substance consisting of an aromatic compound containing at least two non-adjacent hydroxyl groups, with a phosphorylating agent each of said coupling substance and said steroid being introduced into the phosphorylation reaction at any stage of said reaction and the reaction continued until said high-molecular weight phosphorus-containing derivatives being non-dialysable and soluble in aqueous alkali are secured, the reaction mixture being subsequently hydrolysed.

By varying the conditions under which the phosphorylation takes place, products may be obtained, having different molecular sizes, whereby it is possible to get a more or less protracted effect of the compounds. Generally, the reaction is expediently carried through at temperatures below 0°C, but for tardy reactions it may be expedient to increase the reaction temperature up to the neighbourhood of 100°C, if desired.

According to the invention it is expedient to continue the treatment with phosphorylating agent until the molecular weight of the products is above 2000, since this will result in a completely satisfying protracted effect of the products.

When the reaction has reached a suitable stage, which may be controlled for instance by testing the dialyzability of the product, the reaction is stopped by hydrolysing the reaction mixture for instance by adding crushed ice thereto or by pouring the reaction mixture into ice water.

According to a particularly satisfactory method of carrying out the invention, the steroid is treated with an amount of phosphorylating agent not substantially exceeding that equimolar to the steroid or to the steroid plus the coupling substance.

By limiting the amount of phosphorylating agent in this manner, the formation of chain-shaped molecules is promoted, since the single molecules of the phosphorylating agent thereby have greater possibilities of simultaneously reacting with different molecules of the steroid, the coupling substance, or with both.

As it appears from this description of the method, it is a condition for its accomplishment that the steroid contains one or more hydroxyl groups or enolizable keto groups, since steroids not containing such groups cannot be phosphorylated.

A diester of estradiol with phosphoric acid is known, but retains its hormonal effect only for a relatively short time after injection in the organism, just as the esters mentioned above under group (a).

A monoester of estradiol with phosphoric acid is also known, but has a lower estrogenic effect than estradiol itself. In view of these facts it could not be foreseen that high-molecular compounds of steroids, in which the steroid hormone groups are condensed by esterification with phosphorus acid groups, would show a high hormonal effect. On the contrary, it might be expected that they would only have a low hormone effect, just as the monophosphate of estradiol. Accordingly, the possibility of attaining a considerable—possibly even a very high—protracted effect could still less be foreseen from these known facts.

Preferably, phosphorus oxyhalide, phosphorus trihalide or thiophosphoryl halide, particularly chloride, is used as a phosphorylating agent, but other such agents may also be used, if desired, in combination with the above, for instance phenylphosphoryl dichloride.

It is assumed that the protracted effect of the novel compounds results from the compounds being decomposed piecemeal in the organism, presumably by the action of enzymes. In some cases, it may be expedient to be able to regulate this decomposition, and it is an object of the invention to provide the possibility for such a variation of the speed, with which the steroid is placed at the disposal of the organism. In order to attain this object, the steroid may be phosphorylated

and coupled with a special coupling substance through the phosphorus acid group or groups. This makes it possible to build up high-molecular compounds in which only some of the groups, which are linked together by phosphorus acid groups, are of steroid character, for instance every second group, so that the liberation of the hormone is correspondingly delayed. Substances built up in this manner, therefore, are also comprised by the invention.

According to the invention, the coupling substance may be a di- or polyphenol wherein the hydroxyl groups, if there are only two, are not adjacent. Examples of such substances are phloroglucinol, phloretin and phloridzin. Moreover, many other natural or synthetically produced substances of similar character exist which are suitable as coupling substances in the present compounds. Some of these are mentioned in British Patent Specification No. 753319, and high-molecular weight compounds, in which these coupling substances form part together with steroids, are also objects of the present invention.

The use of a coupling substance makes it further possible to build up high-molecular weight compounds from steroids having only one hydroxyl group or enolizable keto group in the molecule, whereas, if no coupling substance is used, the steroid in question should have at least two groups in the molecule in order that the chain formation necessary for producing high-molecular compounds may be attained.

According to an expedient embodiment of the invention a steroid with only one hydroxyl group or enolizable keto group in the molecule is coupled by phosphorylation to a substance with at least three or hydroxyl groups or both kind of groups, whereby molecules of the said substance are coupled together by means of phosphorylation before, during or after coupling with the steroid-containing group. Thus, two of the hydroxyl groups of the coupling substance serve for the building-up of a molecule in which the links are connected by means of phosphorus acid groups and a third hydroxyl group serves to bind the steroid through the phosphorus acid group, whereby the steroid is coupled to the molecule. High-molecular weight compounds built up in this manner are within the scope of the invention.

Practically, this embodiment of the invention may be carried out by first phosphorylating the steroid with the equimolar amount of or, preferably, with a slight excess of the phosphorylating agent. If phosphorus oxychloride is the phosphorylating agent, compounds are formed of the type $ROP(O)Cl_2$, wherein R represents the steroid group. Then the coupling substance is added, whereby either diesters are formed of the type $ROP(O)(Cl)OR_1$, wherein R_1 is a group derived from the coupling substance. Then a further amount of phosphorylating agent is added, and, since R_1 contains at least two hydroxyl groups which are not involved in the diester or esteramide formation, a coupling with phosphorus acid groups may take place at suitable conditions of reaction, resulting in high-molecular weight compounds. It is also possible to proceed in such manner that the steroid and the coupling substance are directly mixed, whereafter the mixture is phosphorylated, or the coupling substance may first be phosphorylated, and then the phosphorylated steroid, or non-phosphorylated steroid together with phosphorylating agent, is added.

According to the invention, an agent promoting condensation may be used, such as a tertiary amine, in order to promote the building-up of high molecular compounds. Examples are pyridine and quinoline. Sometimes the condensation agent may also serve as a reaction medium, but often it will be expedient to use an indifferent solvent, such as ether, dioxane or acetone.

Examples of steroids, which may be phosphorylated directly to high-molecular weight compounds and which, accordingly, contain at least two hydroxyl groups or keto groups which form hydroxyl groups by enolization, are estradiol, methylandrostenediol, estriol, testosterone, pregnanediol and digitoxigenin. Examples of steroids having only one hydroxyl group or keto group which may be enolized to form a hydroxyl group, and which may accordingly be built up to high-molecular weight compounds by means of a coupling substance, are estrone, progesterone, and 19-nortestosterone. High-molecular compounds of these steroids with phosphorus acids and, in the case of most hydroxyl steroids with a coupling substance are within the scope of the invention, as well as the corresponding compounds of other similar steroids.

As mentioned above it is characteristic of the compounds according to the invention that they consist principally of molecules, in which the single groups are linked together by phosphorus acid groups, and wherein the steroid groups are linked together through phosphate, phosphite or their phosphate groups.

The compounds according to the invention are soluble in water at neutral or alkaline reaction and are fairly stable against hydrolysis.

In the simplest case, that in which the steroid forming part of the compounds has at least two hydroxyl groups, including hydroxyl groups resulting from the enolization of keto groups, the compounds of the invention consists of steroid groups linked together by phosphorus acid groups.

As mentioned above, it may be desirable to

produce compounds which, for a given molecular weight, contain less steroid than do the simplest compounds. As mentioned, this can be obtained by using a coupling substance, and according to the invention compounds of this kind consist of molecules, wherein steroid groups and groups derived from di- or polyphenols with at least two non-adjacent hydroxyl are linked together with phosphorus acid groups. The amount of steroid in these compounds is dependent on the molar proportion between steroid and coupling substance and may be varied by modifying this proportion.

In the case of steroids with only one hydroxyl group or enolizable keto group in the molecule, a coupling substance is used to build up the high-molecular weight compounds, and according to the invention such compounds consists of molecules, wherein the phosphorus acid groups link together groups which are derived from polyphenols with at least three hydroxyl groups in the molecule, to which latter groups the steroid is linked through phosphorus acid groups. The latter groups may hereby be linked to a single, to several or to all of the links bound together by phosphorus acid groups in the molecule, all in accordance with the proportions in which steroid and coupling substance are used.

In order to obtain the desired prolonged effect when used therapeutically, the compounds of the invention should have a size making them non-dialyzable. By a molecular weight of 2000 or above that number, the compounds are non-dialyzable if suitable dialysis membranes are used. During the preparation, this gives the possibility of eliminating compounds with lower molecular weight not having the desired prolonged effect.

A prolonged effect of steroid hormones is most easily substantiated in animal tests. When testing the strength of estrogens, the so-called vaginal smear technique is used, i.e. the duration of vaginaloestrus in the test animals (castrated mice) is determined.

By investigations of the strength of androgens, the increase in weight of the prostata may be used, and by testing the anabolic effect, the weight increase of the levator ani may be used as a measure.

The result of such tests is illustrated in the accompanying drawings, wherein

Figure 1 contains the curves A, B, C and D showing the effect of injections of estradiol benzoate, ethinyl estradiol, estradiol phosphate and a polyestradiol phosphate according to the invention, respectively,

Figure 2 contains the curves I, II and III, illustrating the effect of injections of testosterone propionate in oil, and of a polytestosteronephloretin phosphate according to the invention, in comparison with control tests, and

Figure 3 contains the curves I and II showing the effect of a polymethylandrostenediol phosphate according to the invention, and of testosterone propionate in oil, respectively, as well as a control test represented by the point III.

In Figure 1 the ordinate of the curves represents the percentage of test animals which showed response to the hormone injection, and the abscissa gives the time in days after the injection. As a measure of the effect of the hormone, there is used the number of days in which at least 50% of the test animals display vaginal oestrus. The 50% border is shown in dotted lines on the graphs of Fig. 1.

In the case of estradiol diphosphate, 200 micrograms were used for the injection, and in the case of the other products 20 micrograms were used, dissolved in equal amounts of propylene glycol. It appears from the curves that the effect of the estradiol benzoate, the ethinylestradiol and the estradiol diphosphate injections has a duration of about 4, 4 and 3 days, whereas the effect of the polyestradiol phosphate has about 26 days duration. This shows without question that the estradiol is liberated continuously and very slowly from the polyestradiol phosphate, which is exactly the effect which should be aimed at in order to approach natural hormone production as closely as possible.

Corresponding tests have been made with polyestronephloretin phosphate with quite similar results, and clinical tests have further confirmed the results obtained in the animal tests.

In Figure 2, curve I shows the weight of the ventral prostata at different times after administration to castrated rats of 3,75 mg of testosterone propionate in oil, curve II illustrates corresponding tests with 3,75 mg of polytestosteronephloretin phosphate, and curve III represents untreated control animals, the ordinate giving the weight in milligrams of the ventral prostata, and the abscissa giving the number of days after the injection. Each point on the curves represents five animals.

The curves show that with polytestosteronephloretin phosphate a very uniform and strongly prolonged effect is obtained. Even if the supplied amount of testosterone in the form of polytestosteronephloretin phosphate is only half of that supplied as testosterone propionate, and even if the preparation according to the invention is supplied as an aqueous solution, whereas the propionate is supplied in oil depot, the hormonal effect of the preparation according to the invention exceeds that of the testosterone propionate after 16 days.

In Fig. 3, curve I shows the effect on the increment of levator ani in castrated rats of polymethylandrostenediol phosphate admin-

istered in aqueous solution, curve II shows the effect of the same dose of testosterone propionate in oil, and the point III represents a control test. The ordinate gives the increment of levator ani in milligrams per 100 grams of test animals, and the abscissa the amount of administered hormone preparation in milligrams, each point on the curves representing five animals, and the determinations having been made on the 13th day after the injections. It appears from the experiment that polymethylandrostenediol phosphate has a strong and very prolonged anabolic effect.

That there is no question of a depot effect at the place of injection, as in the known preparations with prolonged effect, has been shown by an experiment, where a phosphorylating agent containing radioactive phosphorus (P^{32}) was used for the production of the products in accordance with the invention. These tests have shown that the high-molecular weight acid of phosphorus-compounds are retained in the organism, as, for example, in the blood and the liver. Because of their high molecular weight and their polyanionic character, it is likely that the components are coupled to proteins in the organism.

The invention can be illustrated by the following examples.

Example 1.

Three grams of estradiol are dissolved in 75 ml of anhydrous pyridine. The solution is cooled to —10°C, whereupon a solution of 1.1 ml of phosphorus oxychloride in 10 ml of anhydrous pyridine is added with shaking. After the addition, which requires 7 minutes, the reaction mixture is kept at —10°C for a further period of 3 hours, and then it is left standing at room temperature for 15 hours. A clear solution thus results, to which crushed ice is added. The resulting solution is evaporated in vacuum to dryness. After drying in a vacuum desiccator, 3.8 g of a white powder are obtained. This powder is suspended in 2 ml of pyridine, and 25 ml of 0.5-normal sodium hydroxide are added whereby a solution is obtained, which is then diluted with water to 100 ml. The solution is then dialysed through a Cellophane (Registered Trade Mark) membrane against 4 liters of water for 10 hours with stirring. The dialysis is twice repeated with fresh amounts of water. To the dialysed solution, 2 ml of 1-normal hydrochloric acid are added whereby polyestradiol phosphate is precipitated as a white bulky precipitate. This is centrifuged off and washed repeatedly with 0.1-normal hydrochloric acid. Thereafter it is dried in a vacuum desiccator. The yield is 3 g of polyestradiol phosphate. The analysis shows 0.65% of humidity, 1.35% of pyridine and 9.3% of phosphorus (calculated on a dry sample).

Example 2.

0.55 g of methylandrostenediol are dissolved in 15 ml of anhydrous pyridine. The solution is cooled to —10°C whereafter a solution of 0.18 ml of phosphorus oxychloride in 5 ml of anhydrous pyridine is added with shaking. The addition lasts 3 minutes. The reaction mixture is left standing and hydrolyzed as in example 1. The recovered solution is evaporated and gives after drying in a vacuum desiccator 0,65 g of a yellow powder. This contains 60% non-dialyzable polymethylandrostenediol phosphate.

Example 3.

0.016 g of estriol are dissolved in 0.8 ml of anhydrous pyridine. The solution is cooled to —15°C, whereafter 0.55 ml of a solution of 0.5 ml phosphorus oxychloride in 50 ml of anhydrous pyridine are added. The reaction mixture is left standing for 5 hours at —5°C and then worked up as in example 2. The yield is 0.0125 g of polyestriol phosphate.

Example 4.

0.5 g of testosterone are dissolved in 10 ml of anhydrous pyridine. At —10°C the solution is added dropwise with shaking to a solution of 0.19 ml of phosphorus oxychloride in 6 ml of anhydrous pyridine. The addition lasts 4 minutes. The mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking and cooling to a solution of 0.47 g of phloretin in 5 ml of anhydrous pyridine. The addition lasts 2 minutes. The mixture is left standing at —10°C for ½ hour, whereafter a solution of 0.15 ml of phosphorus oxychloride in 5 ml of anhydrous pyridine is added dropwise with shaking. The addition lasts 3 minutes. The mixture is left standing at —10°C for 3 hours and then at room temperature for a further 15 hours. Then finely crushed ice is added to the mixture and, after filtration of a minor amount of undissolved substance, the solution is evaporated in vacuum to dryness. The residue is dissolved in 10 ml of 2-normal sodium hydroxide and precipitated with 15 ml of 2-normal hydrochloric acid saturated with sodium chloride. The product is filtered and washed with saturated sodium chloride solution and then dried in a vacuum desiccator. The yield is 1.8 g, and the product contains 75% non-dialyzable polytestosteronephloretin phosphate.

Example 5.

0.251 g of testosterone are dissolved in 5 ml of anhydrous pyridine. At —10°C, the solution is added with shaking to a solution of 0.095 ml of phosphorus oxychloride in 3 ml of anhydrous pyridine. The addition lasts 2 minutes. The mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking and cooling to a solution of 0.11 g of phloroglucinol in 2.5 ml of anhydrous pyridine. The addition lasts 2 minutes. The mixture is left standing at

—10°C for 1 hour, whereafter a solution of 0.075 ml of phosphorus oxychloride in 2.5 ml of pyridine is added with shaking. The mixture is then left standing at —10°C for 3 hours, and at room temperature for a further 15 hours. Then finely crushed ice is added, and the resulting solution is evaporated in vacuum to dryness, whereafter it is worked up as in example 4. The yield is 0.33 g, and the product contains 93% non-dialyzable polytestosteronephloroglucinol phosphate.

Example 6.

0.235 g of estrone are dissolved in 5 ml of anhydrous pyridine. The solution is added with shaking to a solution of 0.095 ml of phosphorus oxychloride in 3 ml of anhydrous pyridine. The addition lasts 3 minutes, and the mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking and cooling to a solution of 0.11 g of phloroglucinol in 2.5 ml of anhydrous pyridine. The addition lasts 2 minutes. The treatment is continued in the same manner as in example 4. The yield is 0.35 g, and the product contains 91% non-dialyzable polyestronephloroglucinol phosphate.

Example 7.

0.47 g of estrone are dissolved in 10 ml of anhydrous pyridine. The solution is added with shaking to a solution of 0.18 ml of phosphorus oxychloride in 6 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 4 minutes. The mixutre is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking to a solution of 0.47 g of phloretin in 5 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 3 minutes. The mixture is left standing at —10°C for ½ hour, whereafter a solution of 0.15 ml of phosphorus oxychloride in 5 ml of anhydrous pyridine is added with cooling and shaking. The mixture is left standing at —10°C for 1½ hours, whereafter finely crushed ice is added. A little precipitate, which does not dissolve, is filtered off, and the filtrate is evaporated in vacuum to dryness, whereafter it is worked up in the manner described in example 4. The yield is 1.05 g, and the product contains 95% non-dialyzable polyestronephloretin phosphate.

Example 8.

0.235 g of estrone are dissolved in 5 ml of anhydrous pyridine. The solution is added with shaking to a solution of 0.095 ml of phosphorus oxychloride in 3 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 2 minutes. The mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking to a solution of 0.264 g of quercetin in 2.5 ml of anhydrous pyridine which is cooled to —10°C. The addition again lasts 2 minutes. The mixture is left standing at —10°C for 1 hour, whereafter a solution of 0.075 ml of phosphorus oxychloride in 2.5 ml of anhydrous pyridine is added with continued cooling and shaking. The addition lasts 2 minutes. The mixture is left standing at —10°C for 3 hours, whereafter finely crushed ice is added. The resulting solution is evaporated in vacuum to dryness and worked up as described in example 4. The yield is 0.65 g and the product contains 95% non-dialyzable polyestronequercetin phosphate.

Example 9.

0.235 g of estrone are dissolved in 5 ml of anhydrous pyridine. The solution is added with shaking to a solution of 0.095 ml of phosphorus oxychloride in 3 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 2 minutes. The mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking to a solution of 0.37 g of phloridzin (anhydrous) in 5 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 2 minutes. The mixture is left standing at —10°C for 1½ hours, whereafter a solution of 0.12 ml of phosphorus oxychloride in 4 ml of anhydrous pyridine is added with continued cooling and shaking. The addition lasts 2 minutes. The mixture is left standing at —10°C for 2 hours, whereafter finely crushed ice is added. The working up proceeds then as described in example 4. The yield is 1.1 g, and the product contains 94% non-dialyzable polyestronephloridzin phosphate.

Example 10.

0.235 g of estrone are dissolved in 5 ml of anhydrous pyridine. The solution is added with shaking to a solution of 0.095 ml of phosphorus oxychloride in 3 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 2 minutes. The mixture is left standing at —10°C for ½ hour, whereafter it is added dropwise with shaking to a solution of 0.53 g of rutin (anhydrous) in 5 ml of anhydrous pyridine which is cooled to —10°C. The addition lasts 2 minutes. The mixture is left standing at —10°C for 1½ hours, whereafter a solution of 0.19 ml of phosphorus oxychloride in 6 ml of anhydrous pyridine is added with continued cooling and shaking. The addition lasts 3 minutes. The mixture is left standing at —10°C for 15 minutes, whereafter finely crushed ice is added. The working up proceeds as in example 4. The yield is 0.6 g, and the product contains 90% non-dialyzable polyestronerutin phosphate.

Example 11.

0.25 g of estradiol are dissolved in 10 ml of anhydrous pyridine. At —10°C a solution of 0.13 ml of thiophosphoryl chloride in 5 ml of anhydrous pyridine is added with cooling and shaking. The addition lasts 5 minutes. The mixture is left standing at —10°C for 3 hours and then at room temperature for further 15 hours. Then finely crushed ice is added,

whereafter the resulting solution is evaporated in vacuum to dryness. The residue is ground with 2-normal hydrochloric acid, and then filtered and washed with water and finally dried in a vacuum desiccator. The yield is 0.3 g, and the product contains 95% non-dialyzable polyestradiol thiophosphate.

Example 12.

0.272 g of estradiol and 0.11 g of resorcinol are dissolved in 5 ml anhydrous pyridine. A solution of 0.19 ml of phosphorus oxychloride in 5 ml of anhydrous pyridine is added at —10°C with shaking. The addition lasts 3 minutes. The mixture is left standing at —10°C for 3 hours and then at room temperature for a further 15 hours. Then finely crushed ice is added, and after 24 hours standing a clear solution is formed which is evaporated in vacuum to dryness. The product is dissolved in 10 ml of 1-normal sodium hydroxide solution and precipitated with 5 ml of 5-normal hydrochloric acid. The precipitate is filtered off and washed with a few milliliters of water, whereafter it is dried in a vacuum desiccator. The yield is 0.5 g and the product contains 96% of non-dialyzable polyestradiolresorcinol phosphate.

Example 13.

0.55 g of estradiol are dissolved under heating in 5 ml of anhydrous dioxan. Then 0.19 ml of phosphorus oxychloride are added, and the solution is heated on a steam bath with reflux. After 17 hours, an amorphous mass is formed. The mixture is cooled and 50 ml of water are added. A precipitate is formed which is filtered off, washed with water and dried in a vacuum desiccator. The yield is 0.71 g and the product contains 75% of non-dialyzable polyestradiol phosphate. The analysis shows 3.1% of humidity and 8.2% of phosphorus in a dried sample.

Example 14.

0.3 g of testosterone are dissolved in 10 ml of anhydrous pyridine. At 20°C 0.11 ml of phosphorus oxychloride are added in one portion. The mixture is left standing for 48 hours at room temperature, and then heated on a steam bath for 1 hour. Afterwards it is cooled, and finely crushed ice is added. The resulting solution is evaporated in vacuum to dryness, and the residue is ground with dilute hydrochloric acid. A reddish powder is obtained, the dialysis of which shows that it contains 90% of non-dialyzable polytestosterone phosphate.

Example 15.

0.55 g of estradiol are dissolved in 5 ml of dioxan. After addition of 0.18 ml of phosphorus trichloride the mixture is heated for 25 hours on a steam bath with reflux. After cooling, finely crushed ice is added whereby a white precipitate is formed. This is filtered off and washed with water. After drying in a vacuum desiccator, 0.66 g of a white powder are obtained. The product contains about 50% of non-dialyzable polyestradiol phosphite. The phosphorus content is 9.3%.

What we claim is:—

1. As new compounds high-molecular weight derivatives of hydroxyl group-containing steroids with sex hormonal effect, including steroids in which the hydroxyl groups result from enolisation of keto groups, said derivatives consisting of molecules, in which the steroid groups present and any aromatic groups which may be present are linked together through phosphate, phosphite or thiophosphate groups, the steroid groups being linked to phosphorus through oxygen atoms and said aromatic groups being linked to phosphorus through oxygen atoms, said high molecular weight steroid derivatives being non-dialysable and soluble in aqueous alkali.

2. The new compounds as defined in claim 1, which consist of steroid groups linked together through phosphate, phosphite or thiophosphate groups.

3. The new compounds as defined in claim 1, consisting of molecules, in which steroid groups and groups derived from di- or polyphenols with at least two non-adjacent hydroxyl groups are linked together through phosphate, phosphite or thiophosphate groups.

4. The new compounds as defined in claim 1, consisting of molecules in which phosphate, phosphite or thiophosphate groups link together groups derived from polyphenols with at least three hydroxyl groups in the molecule to which latter groups the steroid groups are linked through phosphate, phosphite or thiophosphate groups.

5. The new steroid derivatives substantially as described herein.

6. A process of producing high-molecular weight phosphorus-containing derivatives of hydroxyl group containing steroids with sex hormonal effect, including steroids with hydroxyl groups resulting from the enolisation of keto groups, in which steroids, having sex hormonal effect and having at least two hydroxyl groups in the molecule, or mixtures of hydroxyl-containing steroids having sex hormonal effect including steroids with hydroxyl groups resulting from the enolisation of keto groups with a coupling substance consisting of an aromatic compound containing at least two non-adjacent hydroxyl groups, are reacted with a phosphorylating agent, introducing each of said coupling substance and said steroid into the phosphorylation reaction at any stage of said reaction and continuing said reaction until said high-molecular weight phosphorus-containing derivatives being non-dialysable and soluble in aqueous alkali are secured, the reaction mixture being subsequently hydrolysed.

7. A process as defined in claim 6, in which the amount of the phosphorylating agent

does not substantially exceed that equimolar to the amount of the steroid or of the steroid plus the coupling substance.

8. A process as defined in any of claims 6 to 8, in which the coupling substance is a di- or polyphenol.

9. A process as defined in any of claims 6 to 8, in which steroid molecules containing only a single hydroxyl group or a hydroxyl group resulting from the enolisation of a keto group, are reacted with a phosphorylating agent in the presence of a coupling substance containing at least three hydroxyl groups, whereby the molecules of the said coupling substance are linked together by phosphorylation before, during or after coupling with the steroid-containing group.

10. A process as defined in any of claims 6 to 9, in which the phosphorylation is carried out in the presence of a hydrogenhalide binding agent, such as a tertiary amine.

11. A process of producing steroid derivatives substantially as herein described with reference to the Examples.

12. Steroid derivatives as claimed in any of claims 1 to 5 when prepared as claimed in any of claims 6 to 11.

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London, W.C.2.

Hastings: Printed for Her Majesty's Stationery Office, by F. J. Parsons, Ltd., 1957.
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Fig. 1.

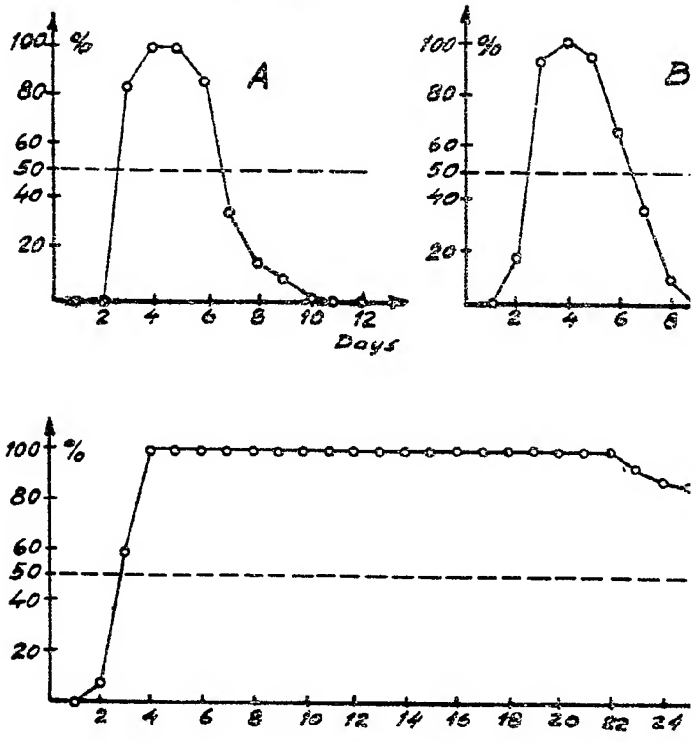
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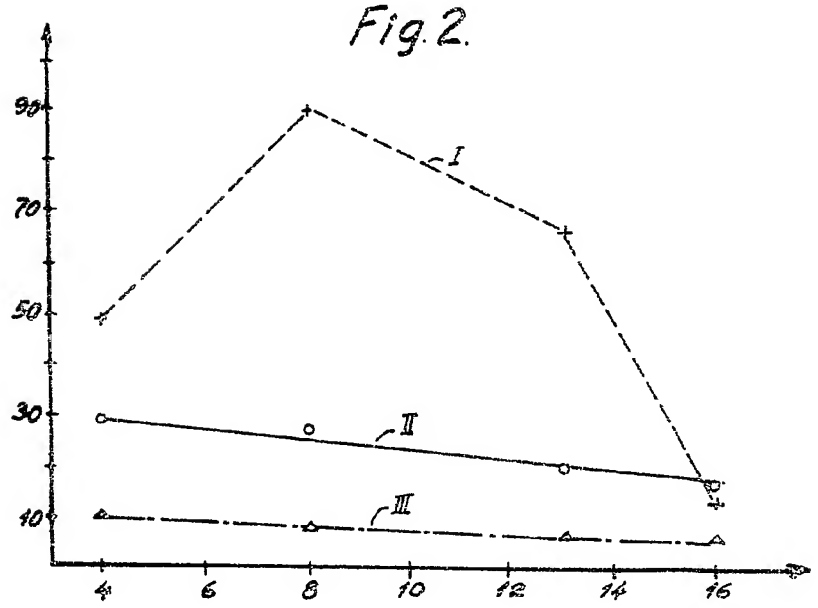
Fig. 2.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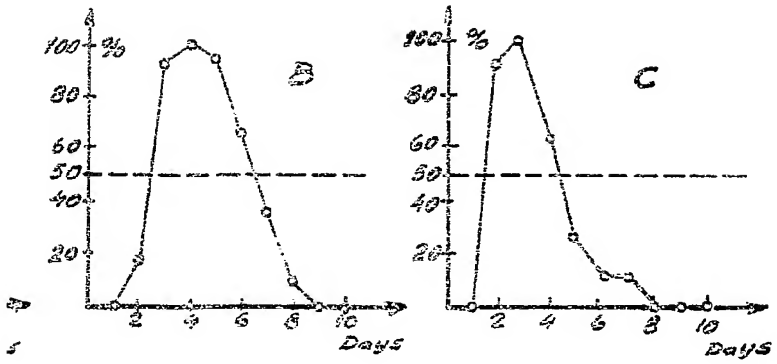
778,142 COMPLETE SPECIFICATION
2 SHEETS

This drawing is a reproduction of the Original on a reduced scale.
SHEETS 1 & 2



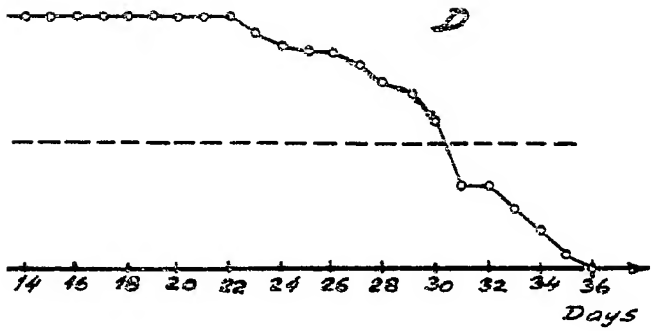
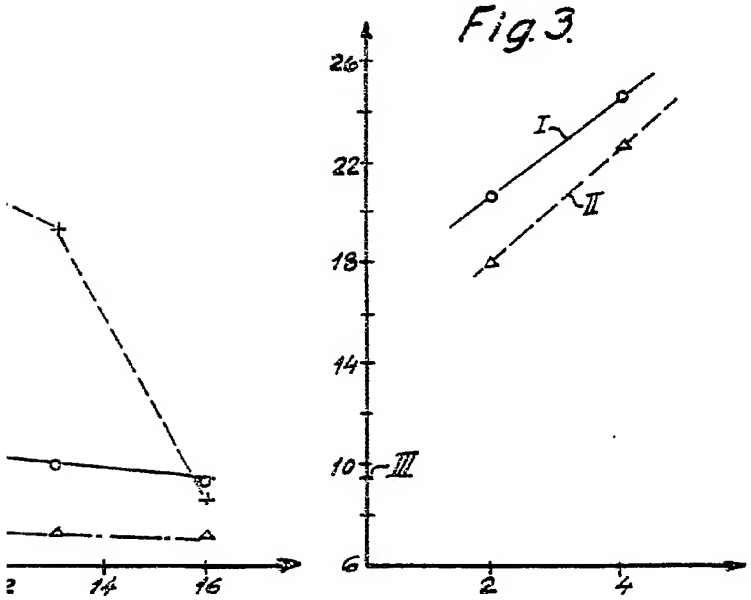


Fig. 3.



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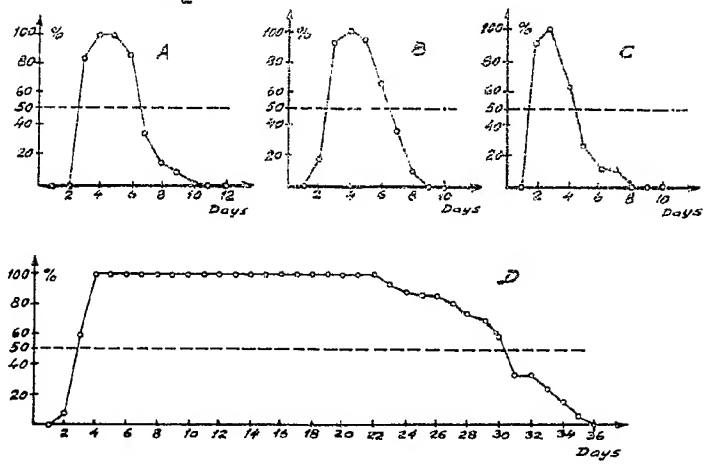

Fig. 2.

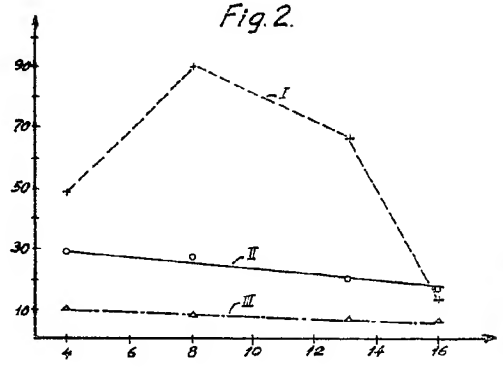

Fig. 3.